

THE TOXICOLOGICAL IMPORTANCE OF LEAD IN BONE: THE EVOLUTION AND POTENTIAL  
USES OF BONE LEAD MEASUREMENTS BY X-RAY FLUORESCENCE TO EVALUATE TREATMENT  
OUTCOMES IN MODERATELY LEAD TOXIC CHILDREN

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INTRODUCTION

Environmental lead exposure, with its concomitant risk of toxicity, remains a major health hazard for children in the United States. Even "low" levels of lead are associated with a wide range of metabolic disorders and with neuropsychological deficits (Angle et al., 1982; CDC, 1985; EPA, 1985; Mahaffey et al., 1982a; Needleman et al., 1979; Piomelli et al., 1982; Rosen et al., 1980; Rosen, 1985). Despite efforts at eliminating lead-based paints and the introduction of lead-free gasoline, an estimated 2.3 to 3.9 million American children between 9 months to 5 years of age have elevated blood lead concentrations ( $>25$   $\mu\text{g}/\text{dl}$ ), according to the new guidelines from the Centers for Disease Control (CDC) (CDC, 1985; Mahaffey et al., 1982a; Yip and Dallman, 1986). Based upon these CDC guidelines, about 9 percent of children in this age range have elevated blood lead concentrations ( $>25$   $\text{mg}/\text{dl}$ ) that require medical intervention. The magnitude of this national health issue in children (rich, poor, white, black, city or country-based) was realized when the results of the Second National Health and Nutrition Examination Survey (NHANES II) were published in 1982 (Mahaffey et al., 1982a). This representative cluster sample defined the incidence of elevated blood lead levels nationally in children of this age; and these data confirmed earlier impressions that blacks and disadvantaged, inner city children were affected disproportionately.

As a result of national screening programs, symptomatic high level lead toxicity has largely disappeared in American children. In the 1980's, this disease is characterized by discrete biochemical and neuropsychological impairments (Angle et al., 1982; CDC, 1985; EPA, 1985; Mahaffey et al., 1982a; Needleman et al., 1979; Piomelli et al., 1982; Rosen, 1985; Rosen et al., 1980) in children who are "asymptomatic" by usual clinical criteria. These biochemical and neuropsychological abnormalities (reviewed elsewhere - Rosen, 1985) are considered to be adverse health effects by the United States Public Health Service (CDC, 1985) and the Environmental Protection Agency (EPA, 1985). Unfortunately, this disease and its adverse health effects are not likely to disappear in the near future. It is currently estimated that over 27 million residential units contain leaded paint at sufficiently high concentrations to be potentially hazardous to children (CDC, 1985). These data suggest that the lead paint problem will be present in the United States for many decades. Even when all such residential units are demolished, a considerable amount of lead paint will remain in the soil of old construction sites.

In view of the high incidence of this disease in American children, and in view of the themes of this conference, the present discussion will focus on the following topics: 1) the limitations of current testing methods to screen, identify and recognize asymptomatic, lead-toxic children who require chelation therapy; 2) the biological importance of lead in bone; 3) the evolution of X-ray fluorescence (XRF) to measure bone lead in vivo; and 4) a conceptual approach to integrate XRF measurements of bone lead in children into a comprehensive clinical research program.

#### **CURRENT LIMITATIONS IN COMMONLY USED METHODS TO SCREEN AND ASSESS LEAD TOXICITY IN CHILDREN**

##### **Erythrocyte Protoporphyrin (EP) Screening**

Screening of American children, unless the situation is unusual (possibility of acute fulminant lead poisoning), is usually carried out first by measurement of erythrocyte protoporphyrin (CDC, 1985). This measurement, using hematofluorometry or more accurate extraction methods (CDC, 1985), is fast, convenient, inexpensive and also has the capability of identifying children who have iron deficiency. Moreover, the volume of blood needed for this determination is small; such volumes can be readily obtained by capillary sampling. Though children may have mildly to moderately elevated blood lead levels without concurrent increases in EP concentrations (see below), and though such children should be identified, it is generally beyond the resources and capabilities of most screening programs to permit both blood lead and EP to be measured at the same time, obtaining a venous blood sample. Capillary samples are prone to environmental contamination with lead (leading to spurious blood lead values); and venous sampling is generally considered to be less acceptable to parents and technically more difficult. Primarily for these compelling and practical public health reasons, initial EP screening is now the cornerstone for identifying lead-toxic children (CDC, 1985).

Nonetheless, there are highly important limitations in the sensitivity of the EP test to detect children who have elevated blood lead levels (Chisolm, 1984; Piomelli et al., 1982). Depending on the series and number of children evaluated, at a blood lead value of 25  $\mu\text{g}/\text{dl}$ , only about 30 percent of children will have an elevated EP concentration (above 35  $\mu\text{g}/\text{dl}$ ) (Piomelli et al., 1982). At blood lead concentrations between 40-49  $\mu\text{g}/\text{dl}$  the response rate (percent of children with elevated EP) is only about 50 percent (Chisolm, 1984). Above a blood lead level of 50  $\mu\text{g}/\text{dl}$ , the response rate finally rises above 90 percent (Chisolm, 1984; Piomelli et al., 1982). It must be recognized, therefore, that despite its practicality, feasibility and cost-effectiveness, the EP test is a relatively insensitive index of lead toxicity until the blood lead value reaches 50  $\mu\text{g}/\text{dl}$ . Moreover, if additional adverse health effects of lead are discerned in the future at blood lead concentrations below 25  $\mu\text{g}/\text{dl}$  (Angle et al., 1982; CDC, 1985; EPA, 1985; Mahaffey et al., 1982a; Piomelli et al., 1982; Rosen et al., 1980), a far more sensitive substitute for the EP test must be found. Even now, significant numbers of children with elevated blood lead values between 25-50  $\mu\text{g}/\text{dl}$  are being missed, when the EP test is used as the primary screening method (Mahaffey et al., 1986).

##### **Blood Lead Values**

In standard pediatric practice, if the EP test is elevated ( $>35 \mu\text{g}/\text{c}$ ) a measurement of blood lead is carried out; this sample must be obtained by venipuncture. Based upon the results of this determination, appropriate medical intervention is instituted (CDC, 1985; Piomelli et al., 1984). It is well recognized that both pediatricians and internists have relied

heavily for decades on the results of this measurement to plan treatment and to assess the toxic effects of lead. It should also be pointed out that this reliance on blood lead measurements is inappropriate. The biological half-time for lead in blood is relatively short and will reflect recent exposure (Chamberlain et al., 1978; Chisolm et al., 1975; Rabinowitz et al., 1973, 1976, 1977). In cases where lead exposure can be reliably assumed to have been at a given level, such as in occupationally exposed adults, a blood lead measurement may be more useful than in instances where some intermittent high level exposure may have occurred, as in children exposed to lead paint. With the development of XRF methodology to measure tibial lead concentration, in tandem with serial blood lead measurements and other biochemical, neurobehavioral and electrophysiological indices, we expect to generate time-concordant blood/bone relationships and determine clearly the previously noted limitations of blood lead values. It is very possible that bone lead content may provide a more reliable index of ongoing tissue lead uptake for population screening, compared to blood lead values. Evidence accumulates in experimental and clinical studies that blood lead values have significant limitations in reflecting both the amount of lead in target tissues (including the brain) and the temporal changes in tissue lead that occur with changes in exposure (Alessio et al., 1976; Azar et al., 1972; Grant et al., 1980; Hansen et al., 1981; Piomelli et al., 1971; Saenger et al., 1982). Moreover, since blood lead levels decline once excessive exposure ends, the blood lead concentration is not indicative of earlier exposure. Furthermore, blood lead concentrations represent a relatively static index of dynamic processes that include intake, equilibration and exchange between body compartments of lead, excretion and tissue sequestration (Rabinowitz et al., 1973, 1976, 1977).

This concept is supported as well by the results of  $\text{CaNa}_2\text{EDTA}$  provocative tests in children in that a significant percentage of children with only mildly to moderately elevated blood lead levels have positive provocative tests (Markowitz and Rosen, 1984; Piomelli et al., 1984). For instance, even at a blood lead concentration between 30-39  $\mu\text{g/dl}$ , about 12 percent of children have positive tests; and at blood lead values between 40-49  $\mu\text{g/dl}$ , almost 40 percent of children have positive tests; this test is considered to be the current reference method for assessing lead stores in children (CDC, 1985; Markowitz and Rosen, 1984; Piomelli et al., 1984). These data suggest the presence of a sizeable pool of bone lead, labile to  $\text{CaNa}_2\text{EDTA}$  mobilization, which is not reflected by blood lead concentrations (Alessio et al., 1976; CDC, 1985; Facchetti and Geis, 1982; Hansen et al., 1981; Markowitz and Rosen, 1984; Rosen, 1985). Moreover, though tissue lead concentrations in children of various ages have been reported (Barry, 1975, 1981), such studies (by design) do not consider relative tissue mass and metabolic state or both in describing lead accumulation with age.

In growing children, the skeletal lead contribution to other tissues is likely governed by skeletal concentration, growth velocity, tissue mass, multiple humoral stimuli, and bone turnover rates (Keller and Doherty, 1980a,b; Marcus, 1983, 1985; Pounds and Rosen, 1986a; Rosen, 1983, 1985; Rosen and Markowitz, 1980; Trotter and Hixon, 1974). Although the overall percentage of bone lead stores is less in children (~75 percent), compared to that in adults (~95 percent) (Barry, 1981), the lead content in bones of growing children increases markedly with age (Barry, 1981; Trotter and Hixon, 1974); it is known that bone mass increases ~40 fold between infancy and adolescence (Trotter and Hixon, 1974), as bone lead concentrations double (Barry, 1981). Thus, skeletal lead accumulation increases by 80-fold. Based upon this marked increase in bone lead during childhood, when skeletal recycling rates are rapid (Rosen and Chesney, 1983), it is reasonable to predict that bone lead will contribute significantly to blood, other soft tissues and the chelatable pool (Chamberlain et al., 1978; Marcus, 1983, 1985; Rabinowitz et al., 1977; Rosen, 1985).

### The CaNa<sub>2</sub>EDTA Provocative Test

To determine if the large numbers of inner city children with blood lead levels between 25 to 55  $\mu\text{g/dl}$  require formal chelation therapy, similar to that in children with blood lead values above 55  $\mu\text{g/dl}$ , the CaNa<sub>2</sub>EDTA test is carried out (CDC, 1985; Markowitz and Rosen, 1984; Piomelli et al., 1984). An 8-hour test has recently been validated and can now be used in children as out-patients (Markowitz and Rosen, 1984). The discussion presented above concerning limitations of blood EP and lead measurements as sensitive indicators of body burden of lead, emphasize the importance of this test to identify those children, who require formal chelation therapy. Stated differently, and others (Markowitz and Rosen, 1984; Piomelli et al., 1984; Rosen, 1985; Saenger et al., 1983) have shown that a significant number of children with elevated body lead stores (defined as blood lead values of 25-55  $\mu\text{g/dl}$ ) have relatively similar increases in chelatable lead as children with higher blood lead toxicity. We consider CaNa<sub>2</sub>EDTA testing to be an essential test to categorize children, who require formal chelation therapy, despite only mild to moderate elevations in blood lead values. In its new guidelines, CDC has placed considerable reliance on this test in such children (CDC, 1985).

Despite our commitment to this testing regimen at our own Center, we recognize that measurements of body lead stores by CaNa<sub>2</sub>EDTA testing is impractical for assessments of large populations of children. Though 8-hour urine collections are not difficult to achieve successfully in young children, comprehensive facilities and resources to conduct this important test in mildly to moderately lead-toxic children are available at too few academic centers (Piomelli et al., 1984).

It is the general theme of this article that XRF measurements of bone lead in children, obtained within a few minutes in an out-patient facility will replace the cumbersome, impractical and expensive CaNa<sub>2</sub>EDTA provocative test. Furthermore, based upon the new detection limits of our XRF instrumentation and future improvements in instrument design, we suggest the real possibility that XRF measurements of tibial lead in children also have the potential for replacing the EP test. In this regard, if our predictions and postulates prove to be correct, elevations in bone lead content measured by XRF may be demonstrable even before other conventional indices of lead toxicity become apparent, such as elevations in EP concentrations.

### THE BIOLOGICAL IMPORTANCE OF LEAD IN BONE

Bone is the major reservoir of body lead stores in adults and children (Barry, 1975, 1981); and it is now recognized that a skeletal sub-compartment of total bone lead is readily exchangeable (Keller and Doherty, 1980a, 1980b; Marcus, 1983, 1985; Rabinowitz et al., 1977; Rosen, 1983, 1985; Rosen and Markowitz, 1980) and therefore a source of endogenous lead available to sensitive soft tissues. Because skeletal lead is a major storehouse (short and long term) of this toxic metal, this source likely contributes to perturbations in essential metabolic pathways together with lead exposure from external sources. In addition, this discussion of the biological importance of lead in bone is pertinent to using and monitoring skeletal stores in children as a proposed index of lead toxicity.

### The Biological Importance of Bone Lead In Vivo

Clinical and experimental studies in vivo have demonstrated an exchangeable bone Pb compartment. Rabinowitz et al. (1973, 1976, 1977) were unable to fully stabilize the isotopic composition of blood Pb with <sup>204</sup>Pb added to the diet of adult humans; and there was a limit to which blood lead isotopically resembled dietary lead. These observations are consistent

in large part, with elegant descriptions of the spatial diffusion of lead into bone matrix (Marcus, 1983, 1985) and with temporal aspects of dose-response relationship of lead in bone. In a series of studies, Keller and Doherty (1980a,b) found that lead transfer from mothers to suckling mouse pups during lactation exceed lead transfer to the fetus from the maternal skeleton. The multiple modulating events of lactation decreased the lead content of the maternal skeleton; and, the role of long-term lead retention in pregnant and non-pregnant mice was governed, in large part, by the rate and amount of lead release from bone (Keller and Doherty, 1980a,b). The latter studies, in conjunction with those demonstrating that the skeleton is the major site of lead chelation by  $\text{CaNa}_2\text{EDTA}$  (Hammond, 1971, 1973), indicate that skeletal lead is of considerable toxicological importance. These concepts are further supported by clinical studies which have shown significant correlations between bone or tooth lead and neuropsychological deficits in children (Needleman et al., 1979; Winneke et al., 1983) and lead nephropathy in adults (Emmerson, 1960; Emmerson and Thiele, 1963).

Based upon recent clinical observations in children (Markowitz et al., 1986; Schwartz et al., 1986), it now appears that the skeleton is also a target tissue to lead. Osteocalcin, a small protein unique to bone, also circulates in blood (Gundberg et al., 1985; Hauschka and Carr, 1982). Osteocalcin is synthesized by osteoblasts, and, once secreted, its three carboxyglutamic acid residues bind calcium ions; and the osteocalcin-calcium structure is subsequently bound to bone hydroxyapatite (Hauschka and Carr, 1982). The circulating pool represents a small fraction of newly synthesized protein which is not adsorbed to bone but released directly into the circulation. Serum levels thereby reflect the amount of bone osteoblastic activity; and serum measurements are considered to be a sensitive indicator of the rate of bone turnover (Gundberg et al., 1985). It has been suggested, though not absolutely proved, that osteocalcin plays an important role in the mineralization process during bone formation (Hauschka and Carr, 1982). In a recent study of mildly lead-toxic children (blood lead 25-51  $\mu\text{g/dl}$ ), we found a marked rise in serum osteocalcin concentrations compared to age-matched controls ( $p < 0.001$ ) (Markowitz et al., 1986). We postulated that lead may inhibit osteocalcin binding to bone, thereby allowing osteocalcin to enter the plasma at relatively high concentrations (Gundberg et al., 1985; Hauschka and Carr, 1982). To test this hypothesis, we found that lead (5  $\mu\text{M}$  or higher) inhibited the binding of osteocalcin to bone hydroxyapatite in vitro. Furthermore, we have recently observed that lead displaces calcium from osteocalcin; and lead has a greater affinity, compared to calcium, for this calcium binding protein. These results are similar to lead's higher affinity for other calcium-binding proteins, including calmodulin (Fullmer et al., 1985), compared to that of calcium's.

These data provide the first clinical evidence of a direct toxic effect of lead on skeletal metabolism. These data suggest the postulate that lead impairs bone cell function, and, ultimately, bone formation in children. In this regard, recent examination and analysis of 2695 children (aged 6 months to 7 years), as part of the second National Health and Nutrition Examination Survey (NHANES II), demonstrated an inverse relationship between blood Pb (in the range of 5 to 35  $\mu\text{g/dl}$ ) and stature (Schwartz et al., 1986). For each 10  $\mu\text{g/dl}$  increase in blood lead (within the above range), there was a 1.2 cm decrease in height after controlling for multiple variables (Schwartz et al., 1986). Moreover, no threshold for this inverse correlation was found. This relationship does not contradict the association between disadvantaged children (with very "mild" lead exposure) and nutritional deficiencies that are known to enhance lead absorption; nor does this relationship establish the specific ingredient in the microenvironment of bone cells responsible for these toxic effects of lead on skeletal growth. However, studies in children dying from lead encephalopathy and in dogs and monkeys fed lead revealed an impairment in bone formation (osteoblast activity) (Eisenstein

and Kawanoue, 1975; VanMullen and Stadhouders, 1974). In vitro studies in separated bone cell population (osteoclasts and osteoblasts) demonstrate that lead, at relatively low concentrations, perturbs calcium homeostasis and calcium mediated function in osteoclasts (Rosen, 1983, 1985). Taken together, these data indicate that the skeleton is a target tissue for lead toxic effects, in addition to its important role as a short and long-term reservoir of lead stores in humans.

#### Bone Lead Values in Adults and Children: Relationship to Lead Nephropathy and Lead-Induced Neuropsychological Deficits

Bone lead concentrations reported in adults have revealed that such values in cortical bone increase in a linear manner with age (Gross et al. 1975; Steenhout, 1982). In contrast, in vertebral bone, which appears to more readily exchangeable, lead concentrations level off during the fifth decade and subsequently decline (Barry, 1975; Gross et al., 1975). This is largely the result and function of the osteoporotic process. Though there are some age and sex-dependent differences in bone lead concentration, the overall patterns of lead accumulation are very similar in cortical and vertebral bones of adults (Barry, 1975).

Based largely on the studies of Emmerson and co-workers (1960, 1963) Australia, the connection was made between lead nephropathy and excessive urinary excretion of lead following the  $\text{CaNa}_2\text{EDTA}$  provocative test. In addition to this diagnostic testing (including determinations of lead in blood), Emmerson and associates (1960, 1963), linked these indices of lead toxicity to marked increases of lead in cortical bone (Table 1); and the results of these studies differentiated readily bone lead concentrations in "normal" adults compared to those in overly exposed patients with characteristic lead nephropathy (Table 1). In a recent study by Kijewski and Lowitz (1982), bone lead concentrations were measured by flameless atomic absorption spectroscopy; and sampled trabecular bone evidenced a marked increase in lead concentration in adults previously exposed to lead in the work place. Taken together, these and others studies have clearly demonstrated a marked increase in bone lead stores, as an index of cumulative exposure, in adult patients with clinical manifestations of lead nephropathy.

Studies collecting autopsy data in normal children have found lower concentrations of lead in the bones of children compared to those of adult (Barry, 1975, 1981), but, from these studies, which are static in nature, the overall patterns of lead sequestration between cortical and vertebral bone are very similar in both adults and children. Differences in lead content of cortical bone in children (Table 2) reveal the most marked difference in contrast to such values in adults (Table 1) (Barry, 1975, 1981). This is not surprising, because the lead content of cortical bone does increase throughout life and does not appear to reflect different metabolic turnover rates characteristic of a child's growing skeleton.

Pioneering studies were reported in 1974 and 1975 that established zo analyses of children's shed teeth, by electron microprobe and chemical techniques, as a highly sensitive measure of cumulative lead exposure (Needleman et al, 1974; Shapiro et al., 1975). These studies showed that lead is concentrated in circumpulpal dentine; and Strehlow's (1972) data in primates demonstrated that whole tooth and secondary dentine lead content are both dose-related and permanent. Measurement of lead in teeth is equivalent to measuring this metal in cortical bone (tibia, for instance), which reflects cumulative exposure. Measurements of lead in whole teeth provide a very similar index of exposure as dentine lead, though zonal analysis of the latter revealed higher concentrations of lead compared to analyses of whole teeth, in which concentrated lead in the dentine area is

Table 1. Bone Lead Values in Adults (ppm, wet weight)

	"Normals"	Overly Exposed	Clinical Disease
Emmerson and Thiele (1960)	14-27 (cortical)	46-195 (cortical)	Lead Nephropathy
Emmerson and Lecky (1963)	8-16 (cortical)	45-66 (cortical)	Lead Nephropathy
Emmerson (1963)	2-16 (cortical)	52-178 (cortical)	Lead Nephropathy
Gross et al. (1975) (cortical)	14.09 ± 11.22		
4-7 (trabecular)			
Barry (1975) (cortical, males)	23-33 ± 15-22 (cortical, males)	74-85 ± 6-58	Unknown
9 ± 6 (vertebral, males)	30 ± 16 (vertebral, males)		
*Kijewski and Lowitz (1982) *(DRY WEIGHT)	4-28 (trabecular)	26-410 (trabecular) Poisoned by Occupational Exposure Probable Nephropathy	Previously Lead

"diluted" by lower concentrations in other parts of the teeth (Table 2). Development of these indices of lead stores permitted systematic evaluation of neurobehavioral function as related to lead burden in children.

The study of Needleman et al. (1979) used dentine lead to separate children into high and low lead groups. Children in the high lead group showed deficits in I.Q., verbal subtests, speech processing, attention and adaptive classroom behavior (Needleman et al., 1979), compared to the low lead group. This study, therefore, directly linked the body burden of lead indexed by dentine lead levels, to a wide range of neurobehavioral deficit. This study has now been confirmed by several others (Yule and Rutter, 1985). Instead of dentine lead content, Winneke et al. (1983) employed the lead concentration in whole teeth and related such values to neurobehavioral deficits in children. These and other studies, reviewed by Yule and Rutter (1985), definitively connected dentine or tooth lead (i.e., cortical bone) to impairments in cognitive functioning in a "dose-dependent" manner.

It is important to point out that a high correlation between tooth lead and blood lead values would not be expected and has not been found (Ewers et al., 1982). In 83 children, the correlation coefficient (though statistically significant) was only 0.47 between tooth lead and blood lead values. These observations are likely explained by fluctuations in blood lead concentration with changing conditions of exposure. In contrast, tooth or dentine lead values represent a cumulative function of long-term lead intake and exposure. Based upon this discussion, it is reasonable to suggest that measurements of cortical bone in humans will allow detection of cases of increased lead absorption, when other indices have returned to normal.

Though the use of tooth/dentine lead to measure the cumulative body burden of lead was an important discovery, such methods also have practical limitations. It is necessary to wait for teeth to be shed and to rely upon children to save these. Furthermore, dental caries and fillings will lead to spurious results, so that these analyses can be carried out only on healthy teeth. Moreover, the 5-8 year old period is the time when teeth shed; and this precludes the use of teeth to relate lead burden to neurobehavioral deficits in younger children. Lastly, as noted in some studies, a lack of concordance has been observed between different tooth samples. Hence, a non-invasive technique to assess accurately and directly cumulative lead stores in young children would present a major breakthrough.

#### The Metabolism of Bone Lead In Vitro: An Endogenous Toxicological Source Lead In Vivo

The first observations of the in vitro metabolism of lead in bone were carried out using bone organ culture techniques with the radius and ulna from fetal rats (Rosen and Wexler, 1977; Rosen and Markowitz, 1980). The studies showed that at least one sub-compartment of total bone lead was readily mobile; and the metabolism of lead in this sub-compartment(s) was controlled by the same ions and hormones that regulate bone calcium metabolism. Though it appears that these studies provided an initial understanding of the net summation of diverse cellular activity in this complex tissue, the use of organ culture techniques precluded investigation of lead's effects at the cellular level - in osteoclasts and osteoblasts. Accordingly, techniques were adopted that permitted studies of separated bone cell populations, enriched in osteoclastic (OC) or osteoblastic (OB) bone cells, in primary monolayer cultures (Rosen, 1983, 1985; Pounds and Rosen, 1986a,b). These techniques involved the separation of bone cell populations derived from mouse calvariae, by a sequential collagenase digestion.

Table 2. Bone Lead Values in Children (1-9 years of age)  
(ppm, wet weight for cortical and vertebral bone)  
(ppm, dry weight for teeth and dentine)

	"Normals"	Overly Exposed	Clinical Disease
1) Vogt (1930) (USA)	-	527 (cortical)	Fatal Lead Poisoning
2) McKhann and Vogt (1933) (USA)	-	602 (cortical)	Fatal Lead Poisoning
3) Barry (1975) (UK)	1-6 (cortical) 0.01 - 6 (vertebral) (mean: 3.01)	-	-
4) Barry (1981) (UK)	0.16-6.20 (cortical) (mean: 2.76)  0.01-6 (vertebral) (mean: 2.4)	-	-
5) Needleman et al. (1974) (USA)	37-48 (tooth dentine) children in 5 schools; range of mean values measured by ASV	66-208 (range in mean values for children in 9 schools)	"Subclinical"
6) Needleman et al. (1974) (USA)	<10 (tooth dentine) ASV	>20 (tooth dentine)	Neuropsychological deficits
7) Winneke et al. (1983) (FRG)	<4 (whole tooth) Flameless AAS	>10	Neuropsychological deficits

ASV = anodic stripping voltammetry; AAS = atomic absorption spectroscopy

It was found that OC cells accumulated considerably larger amounts of lead compared to OB cells and that increasing medium levels of lead markedly perturbed calcium metabolism in OC cells (Rosen, 1983, 1985). More recently using desaturation kinetic techniques, we have characterized the steady-state kinetic distribution and behavior of lead in primary cultures of OC cells (Pounds and Rosen, 1986a). We defined three intracellular pools of lead containing ~10 percent ( $S_1$ ), 12 percent ( $S_2$ ) and ~78 percent ( $S_3$ ) of total cellular lead (1.2 nmol/mg cell protein) (Pounds and Rosen, 1986a). The results indicated that exchange of lead across the plasma membrane of OC occurs readily; the largest of these compartments ( $S_3$ ) is tentatively identified as mitochondrial lead and the intracellular distribution of lead in OC is similar to that in hepatocytes (Pounds et al., 1982a,b, 1983). In fact, the overall qualitative similarities are remarkable between OC and hepatocytes. In both cell types, 75 percent or more of cellular lead was in  $S_3$ , with the remainder divided almost equally between the more rapidly exchanging compartments,  $S_1$  and  $S_2$ . Of the five rate constants defining the steady-state exchange, only two were statistically different between the two cell types. These similarities suggest that differences in sensitivity of target organs to the toxic effects of Pb may depend more on the specific metabolic roles and function of different cell types than on the distribution and/or metabolism of lead within different cells. The metabolism of lead in OC cells has been summarized into a physiological model (Fig. 1). Though the exact characterization of each compartment is incomplete, the model is relevant and useful to facilitate interpretation of the kinetic data and to aid in the design and interpretation of future studies concerning the regulation of lead metabolism in bone cells by calcitropic hormones and intracellular "messengers". In this regard, using very comparable techniques, we have resolved the intracellular metabolism of calcium in OC cells into three intracellular kinetic pools with half-times of 2, 8 and 80 minutes (Pounds and Rosen, 1986b). In general, similar intracellular pathways were present in OC for both lead and calcium.

Taken together, data at the cellular level, as well as results from clinical research (Chamberlain et al., 1978; Rabinowitz et al., 1973, 1976, 1977) and experimental studies (Keller and Ooherty, 1980a,b), provide considerable support for the concept that hormones and ions modulate lead entry and exit from bone in a dynamic manner similar to that of calcium.

#### X-RAY FLUORESCENCE TO MEASURE BONE LEAD STORES IN VIVO: THE EVOLUTION OF TWO APPROACHES

As a biomedical researcher outside the areas of physics and radiobiology, the following discussion will provide, in summary form, the evolution of two techniques to measure bone lead in vivo. Both K- and L-X-rays are suitable for the analysis of lead in bone by X-ray fluorescence (XRF). To induce K-X-ray fluorescence, the incident radiation must be above the 88 KeV K-absorption edge. Because the L-absorption edge is 15.87 KeV, the incident radiation can be provided by low energy sources. These two measurements complement each other, in the sense that quantitation of K-X-rays provides cross-sectional information of the lead concentration in bone and bone marrow, whereas L-X-rays are a useful measure of the lead content in the bone surface alone.

The first description of an XRF system designed to measure soft L-X-rays of lead, in which such measurements were compared by atomic absorption spectroscopy, was reported by Wielopolski et al. (1983). The relationship between the net number of counts in the lead peaks of the tibia, measured with an  $^{125}\text{I}$  source, and the concentration of lead in bone, determined by flameless atomic absorption spectroscopy, yielded a correlation coefficient of 0.90 ( $p < 0.03$ ); and the detection limit for

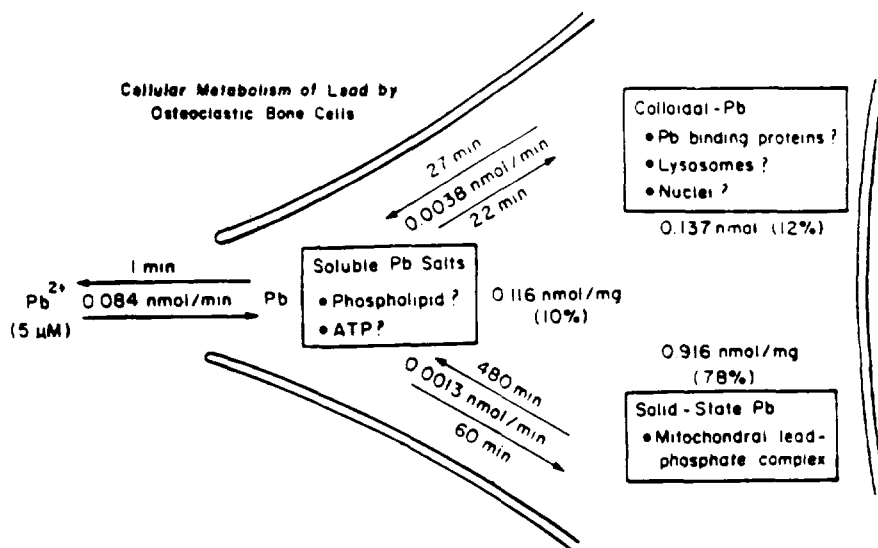


Fig. 1. Physiological model for the steady-state metabolism of lead in cultured osteoclastic bone cells. All data are normalized to one milligram of cell protein, with pool sizes expressed as nanomoles per milligram of protein, fluxes as nanomoles per milligram cell protein per minute and half-times as minutes. Primary cultures of osteoclastic bone cells are triangular in shape (from Pounds and Rosen, 1986).

was 22 ppm or three times the standard deviation of the background (Wielopolski et al., 1983). To further validate these XRF measurements, the solubilized bone samples (used for atomic absorption spectroscopy) were re-analyzed by XRF and a similar *r* value was found (Table 3). The overlying tissue fluctuations were accounted for with a real-time ultrasound unit (7 MHz) that has an accuracy of 0.3 mm. Since cortical bone thickness is more than the saturation thickness of 3.0 mm, the signal was independent of bone density; and a constant volume of bone cortex was sampled by each measurement. It was pointed out that an X-ray machine could also provide a sufficiently intense beam, so that a polarization technique could be utilized to reduce the background and thereby improve the signal-to-noise ratio. Though attenuation of the signal by overlying tissue may be more significant with L-X-rays and though the system is sensitive to changes in geometry, the initial report (Wielopolski et al., 1981, 1983), presented substantive advantages for the use of L-X-rays over K-X-rays. These include: 1) collimation was simplified by measuring bone surface only; 2) the high degree of polarization of low-energy incident radiation provided rationale to improve the detection limits, and 3) the low radiation exposure of this system suggested the possibility of its use in children, in an age-group for which institutional review board approval is difficult to obtain for any radiation exposure that is not immediately diagnostic and/or therapeutic.

This XRF technique, as initially designed, has been used to measure bone lead content in the tibia of 45 industrially exposed workers (Wielopolski et al., 1986a). The mean concentration of lead in bone was observed to be 52.9 ppm; and a significant correlation coefficient (*r* = 0.69, *p* < 0.001) was found between bone lead concentrations and cumulative

Table 3. X-Ray Fluorescence Systems

L-X-Rays	Detection Limits	Comments
Wielopolski et al. (1983)	22 ppm	$^{125}\text{I}$ Source Cadaver Study
Wielopolski et al. (1986a)	20 ppm	$^{109}\text{Cd}$ Source Adult Workers in Industr
Wielopolski et al. (1986b)	~5 ppm	X-ray Tube as Source Polarized Beam  Marked Increase in Detec Limits  Cadaver Study

48-hour urinary lead excretion following administration of  $\text{CaNa}_2\text{EDTA}$ . This finding supports the view, expressed earlier, that XRF methodologies do not have the real potential for replacing the  $\text{CaNa}_2\text{EDTA}$  provocative test. Furthermore, this study reported that a higher percentage of subjects evidenced an elevation in bone lead content (61.4 percent) compared to a significant increase in urinary lead excretion during a  $\text{CaNa}_2\text{EDTA}$  provocative test (38.9 percent). If these data are confirmed in the future, it is reasonable to suggest that a sub-compartment of lead in bone may be inaccessible to  $\text{CaNa}_2\text{EDTA}$  chelation and that such XRF measurements provide the most definitive index of the cumulative body burden of lead.

Very recently, this L-X-ray instrument has been re-designed; and a low-energy X-ray source is now being used to irradiate the tibia (Wielopolski et al., 1986b). The beam has been polarized; and the signal-to-noise ratio has been increased 8 to 9-fold with careful attention to geometry. The new detection limits are approximately 5 ppm with a skin dose of ~0.5 rads and virtually no radiation dose to the bone marrow.

These new detection limits may allow us to measure bone lead concentrations even in normal children. For instance, Ewers et al. (1983) found bone (tooth) lead concentrations in the teeth of normal and smelter children to be 3.9 and 6.0 ppm, respectively, in European cohorts without lead paint exposure. These measurements were carried out by atomic absorption spectroscopy. Using a similar analytical technique in American children, Bellinger et al. (1984) differentiated high from low lead groups on the basis of tooth lead concentrations above and below 10 ppm, respectively. Taken together, these data, relating to marked improvements in L-X-ray detection limits, indicate that it should now be possible to incorporate XRF measurements in lead toxic children into a comprehensive multidisciplinary clinical research protocol (see below); and a new L-X-ray instrument is being installed adjacent to our Pediatric Lead Metabolism Clinic during the summer of 1986.

The first substantive report of the use of the K-X-ray technique in humans (children, specifically) was reported in 1978 (Shapiro et al., 1978). The XRF system could measure tooth lead in excess of 15 ppm with X-ray exposure of about 78 millirads (Shapiro et al., 1978). When tooth lead concentrations were divided by the age of each child, a moderately significant correlation coefficient was observed between tooth lead and

blood lead and EP levels. This "correction" of XRF values assumed that the accumulation of lead in teeth was consistently uniform throughout the lives of children who ranged in age from 2 to 12 years. Though this is unlikely to be a biological reality, 300 urban children were examined in this first reported use of K-XRF in children. Tooth lead values were calculated for three groups of 10 children each, whose blood lead levels ranged from 30-49  $\mu\text{g/dl}$ , 50-79  $\mu\text{g/dl}$  and  $>80 \mu\text{g/dl}$ . The mean tooth lead levels for the three groups were 8.2, 10.4, and 14.8 ppm/year (Shapiro et al., 1978). These values are remarkably high but consistent with those in Philadelphia school children, whose dentine lead values were measured by anodic stripping voltammetry (Needleman et al., 1974).

Ahlgren and co-workers (1980) used the K-XRF methodology to measure bone lead in the phalanges of industrially exposed workers. Of 22 men studied, 15 or 70 percent had lead concentrations in the phalanges above 20  $\mu\text{g/gm}$ , which was the limit of detection; and the lead concentration in the phalanges was found to increase with the length of employment. The absorbed dose at the skin surface was 550 millirads and, in the center of the finger, it was 250 millirads per 40 minutes (Ahlgren et al., 1980). Since the total volume of the finger irradiated was less than 3.5  $\text{cm}^3$ , the total absorbed energy was smaller than that obtained from an ordinary radiograph of the hand.

In Queensland, Australia, forty of 200 adults were found to have elevated bone lead concentrations ( $>25 \text{ ppm}$ ) by a K-XRF technique using  $^{57}\text{Co}$  as a source. This was considered to be a healthy population of adults, some of whom likely had exposure to leaded housepaint as children. No other parameters of earlier lead exposure were included in this study. The most impressive and detailed K-XRF technique was reported by Somervaille et al. (1985). This paper presented a detailed analysis of the measurement technique and of the associated dosimetry, as well as results obtained in adults. Using a  $^{109}\text{Cd}$  source, the minimum detectable lead concentration was 10  $\mu\text{g/gm}$  with an absorbed dose of 10 millirads and a maximum skin dose of 45 millirads. Excellent verification of the XRF measurements was provided by atomic absorption spectroscopy. Moreover, use of this methodology apparently differentiated normal from occupationally exposed adults (Table 4). In the former, the mean tibial lead level was 10  $\mu\text{g/gm}$ , while, in the latter group, the mean value was 31  $\mu\text{g/gm}$  (Somervaille et al., 1985).

To date, instrument development in both L- and K-XRF techniques have characterized the most important papers in this field (Tables 3 and 4), as instrument design has gradually been modified to enhance detection limits. This emphasis on instrument design and development could be anticipated for an evolving technology in its "infancy". Now, however, it appears appropriate to use either or both approaches to evaluate bone lead measurements by XRF within a comprehensive clinical research program.

#### A CONCEPTUAL APPROACH TO INTEGRATE L-XRF MEASUREMENTS OF BONE LEAD IN CHILDREN WITHIN A COMPREHENSIVE CLINICAL RESEARCH FRAMEWORK

Given the recent advances in detection limits for both the L- and K-XRF techniques (Somervaille et al., 1985; Wielopolski et al., 1986a,b), it should now be possible to incorporate the use of either technique to contribute towards clarifying further extant questions in lead toxicology in humans. One such study, to be outlined briefly, is about to begin enrollment of lead toxic children at our Center this summer. Obviously, there are still many questions in lead toxicology to be addressed; and the following summary of our study is only meant to serve as one possible example for the use of XRF methodology within a multidisciplinary clinical research project.

With support from the National Institute of Environmental Health Sciences, we are assessing the biochemical and functional consequences of

Table 4. X-Ray Fluorescence Systems

K-X-Rays	Detection Limits	Comments
Shapiro et al. (1978)	15 ppm	<sup>57</sup> Co Source Measurement of children's teeth in situ
Ahlgren et al. (1980)	20 ppm	<sup>57</sup> Co Source Industrial workers Measurements in phalange
Price et al. (1984)	20 ppm	<sup>57</sup> Co Source 200 Queensland adults Measurements in phalang
Somervaille et al. (1985)	10 ppm	<sup>109</sup> Cd Source Verification by atomic absorption spectroscopy  Differentiation of over exposed vs. normal population of adults

moderate body burdens of lead within a wide-ranging clinical research program that focuses on the effects of chelation treatment. Our multidisciplinary group consists of biomedical scientists in the following fields: pediatric metabolism, child behavior, child development, neuropsychiatry, pediatric epidemiology and biostatistics, and neuroscience. We are interested in relationships between past and present exposure to lead and three types of outcome. Lead exposure is being measured by three methods: 1) blood lead concentrations, an index of recent exposure; 2) urinary lead excretion during CaNa<sub>2</sub>EDTA provocative testing, an index of mobilizable tissue lead stores; and 3) X-ray fluorescence measurements of bone lead, a direct measurement of lead that has accumulated over time. The outcome measures for those affected by lead burden, include: 1) biochemical abnormalities: EP, ALAD in whole blood and 1,25-dihydroxyvitamin D (1,25-(OH)<sub>2</sub>D) and osteocalcin concentrations in serum. We have reported previously that concentrations of this vitamin D hormone are depressed in children over the entire range of blood lead values so far studied, namely 12 to 120 µg/dl (Mahaffey et al., 1982b; Rosen et al., 1980). Moreover, in severely lead toxic children (blood lead >60 µg/dl), 1,25-(OH)<sub>2</sub>D concentrations rose to normal within two days following chelation treatment. Accordingly, measurement of ALAD in blood and 1,25-(OH)<sub>2</sub>D in serum provide dynamic indices of lead exposure that normalize within a different biological time frame compared to EP values; EP levels usually remain elevated for many months following chelation treatment; 2) central nervous system dysfunction evaluated by auditory and somatosensory evoked potentials; and 3) behavioral deficits examined by measures of intelligence, as well as specific measures of attention and reaction time.

We are using a pre-/post-treatment design that will allow us to describe the effects of chelation treatment on multiple outcomes over time to make inferences about the effects of moderate lead toxicity on child brain function and behavior, and to explore different means by which lead may exert its effects.

More specifically, we are examining, in asymptomatic children with moderately increased body lead stores, the short-term (6 weeks) and long-term (6 months) effects of chelation treatment on biochemical indices and electrophysiological and neurobehavioral measures. The effects of treatment will be assessed by the degree of change from pre-treatment levels to post-treatment levels in each outcome variable. We are also investigating the extent to which change in the electrophysiological measures and behavioral indices are correlated with changes in lead burden and biochemical indices of toxicity. Our conclusions about the effects of lead and the efficacy of treatment on functioning will depend upon convergence of results in all three outcome measures, as well as upon a comparison with a nontreated group. In addition, we are comparing the role(s) that different indices of body lead stores have in whatever effects occur with treatment. With the availability of serial measures of lead burden, as well as toxic biochemical effects, we hope to determine what relationship(s) may be present among the different measures of lead toxicity and whether these change over time and with intervention. We also expect to determine which measures or combinations of measures are most associated with behavioral and electrophysiological changes.

These analyses, in turn, will lead to hypotheses about whether brain function and behavior are affected, for example, more by lead circulating in the blood, by cumulative lead exposure over a child's entire life, by secondary results of lead burden, such as increased heme precursors, or by some interaction among the different lead compartments in the body. The analysis will help to establish the validity of the different clinical measures of lead toxicity.

In carrying out this multidisciplinary project, we are testing, hopefully, the following postulates:

1. Initial (pretreatment) scores on both behavioral and electrophysiological measures will be a function of the magnitude of body lead stores, as measured by urinary lead excretion following a single dose of  $\text{CaNa}_2\text{EDTA}$ , and by the previously obtained XRF measure of bone lead.
2. Chelation treatment in children following a positive provocative test will lead to an improvement in standardized cognitive measures, a decrease in reaction time, an improvement in attentiveness, and decreases in latency on the evoked potentials. Some biochemical indices of lead burden ( $1,25\text{-(OH)}_2\text{D}$ , ALAD, osteocalcin) will improve rapidly.
3. Immediate changes are likely to be due to a reduction in chelatable lead. If electrophysiological and behavioral measures are also affected by accumulation of heme precursors, such as protoporphyrin, which are known to recover more slowly after treatment, then further improvement will be seen at the 6 month visit.
4. Treatment of iron deficiency will have an additive effect with chelation treatment, such that children with iron deficiency and increased body lead burdens will show the largest effects of treatment.

Our vigorous efforts to reduce the exposure of children to lead-based paint and to screen and further evaluate those children at risk for such exposure will be strengthened considerably should this study provide evidence for a causal and potentially reversible relationship between body lead stores and cognitive and central nervous system dysfunction. By demonstrating directly the efficacy, or lack thereof, of methods of diagnosis and treatment recommended currently, we are hopeful that a more substantively reasoned approach will be developed for the management of children with "low" level lead toxicity.

## SUMMARY

Based on the previous discussion, it appears that conventional indices of lead toxicity in children have significant limitations. These are related to their relative inconvenience ( $\text{CaNa}_2\text{EDTA}$  testing, tooth lead measurements) and insensitivity to reflect the dynamic nature of lead's toxic effects (blood lead and EP). Because the skeleton is increasingly recognized as a toxicologically significant source of endogenous lead, it is important to emphasize that the metabolism of this metal in bone explants as in experimental and clinical studies of skeletal lead homeostasis, is controlled, in part, by the same ions and hormones that modulate calcium homeostasis. Moreover, the steady-state kinetic distribution and various transfer functions for both lead and calcium are remarkably similar in both cultured osteoclastic bone cells (hard tissue) and hepatocytes (soft tissue). Within this context, XRF measurements of lead in bone offer considerable promise to quantitate serially cumulative body burdens of lead in young children and adults. In view of currently available XRF instrumentation, with further refinements in the years to come, this new capability provides a convenient and increasingly precise methodology to measure cumulative lead exposure, as related to biochemical, electrophysiological and neurobehavioral indices of lead toxicity.

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